REMARKS

The foregoing amendment is being offered to provide a sequence listing complying with current rules.

Entry of this amendment to the specification to identify the peptide sequences and the Sequence List, magnetic disc, and declaration as to likeness of the paper copy and magnetic disc by the Examiner is respectfully requested.

The foregoing amendment provides sequence information derived from the parent PCT (PCT/US99/29743) application. This preliminary amendment is being offered to identify the peptide sequences identified in Applicant's specification and invention. Entry of this amendment by the Examiner is respectfully requested. Attached hereto is a marked-up version of the changes made to the specification by adding the identification of the "Sequence Identification Number (SEQ ID NO:)" by the current amendment. The attached paper is captioned "**Version with Markings to Show Changes Made**". Attached hereto are substitute pages of the patent specification with of the changes made to the specification by adding the identification of the "Sequence Identification Number (SEQ ID NO:)" by the current amendment. The attached paper is captioned by a coversheet "**Substitute Pages**".

Authorization is given to charge payment of any additional fees required, or credit any overpayment, to Deposit Acct. 13-4213. A duplicate of this paper is enclosed for accounting purposes.

Respectfully submitted,

Dated: April 22, 2002

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"**Substitute Pages**"

throughout the specification and in the claims. The primary applications of this invention involve human patients, but this invention may be applied to laboratory, farm, zoo, wildlife, pet, sport or other animals. The products of this invention may optionally employ radionuclide ions, which may be used for diagnostic imaging purposes or for radiotherapeutic purposes.

B1

Peptide and Metallo-Construct Molecule Libraries and Combinatorial Chemistries. Using the methods of this invention, libraries of peptides and peptidomimetics are designed wherein each constituent library member includes an MBD sequence necessary for providing a coordination site for complexation with a metal, it being understood that such sequence may differ among the constituent members of the library. Upon complexing the MBD with a metal, a specific structure results, forming a mimic of a reverse turn structure. The specific stereochemical features of this complex are due to the stereochemistry of the coordination sphere of the complexing metal ion. Thus the preferred geometry of the coordination sphere of the metal dictates and defines the nature and extent of conformational restriction.

Libraries of this invention contain constituents which are either locally or globally constrained structures. Libraries may include molecules with either local conformation restrictions or global conformation restrictions, or some combination thereof. This aspect of the invention includes a variety of methods of synthesis, screening and structural elucidation of positive hits in screening systems. The importance of these aspects are well known to those skilled in the art and will also become evident from the following description and examples.

In general, most of the metals that may prove useful in this invention have a coordination number of 4 to 6, and rarely as high as 8, which implies that the putative MBD must be made of residues with reactive groups located in a stereocompatible manner so as to establish a bond with a metal ion of given geometry and coordination sphere. Coordinating groups in the peptide chain include nitrogen atoms of amine, amide, imidazole, or guanidino functionalities; sulfur atoms of thiols or disulfides; and oxygen atoms of hydroxy, phenolic, carbonyl, or carboxyl functionalities. In addition, the peptide chain or individual amino acids can be chemically altered to include a coordinating group, such as oxime, hydrazino, sulfhydryl, phosphate, cyano, pyridino, piperidino, or morpholino groups. For a metal with a coordination number of 4, a tetrapeptide amino acid sequence may be employed (such as Gly-Gly-Gly-Gly) (SEQ ID NO:1), or a tripeptide amino acid sequence in which at least one of the amino acids has a side chain with a coordinating group can similarly be employed (such as Gly-Gly-Cys). The side chain can have a nitrogen, oxygen or sulfur-based coordination group. Thus, an amino acid sequence can provide an N_4, N_3S, N_2S_2, NS_3, N_2SO or similar ligand, yielding tetradentate coordination of a metal ion utilizing nitrogen, sulfur and oxygen atoms.

In another embodiment of the invention, the MBD includes one or more amino acid residues and one or more derivatized amino acids or spacer sequences, with the derivatized amino acid or spacer

six equivalent of diisopropylethylamine (DIEA) were added with NMP and bubbled with nitrogen for 30 min. The resin was again washed and deprotection of the Fmoc-group repeated, yielding NH_2-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. The resin was then split into two pools, and using similar methods Fmoc-L-Gln-(Trt)-OH was added to one pool and Fmoc-D-Gln-(Trt)-OH added to the other pool. The two pools were mixed and the Fmoc-group deprotected, yielding NH_2-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. The resin was again split into two pools, and Fmoc-L-Asn-(Trt)-OH added to one pool and Fmoc-D-Asn-(Trt)-OH added to the other pool. The two pools were mixed and the Fmoc-group deprotected, yielding NH_2-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. To this resin was added 0.072 g of succinic anhydride in pyridine, yielding $HOOC(CH_2)_2CONH$-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. After washing, the S^tBu group was removed by adding 13.8 mL of DMF/Tributylphosphine (20/3, v/v; 0.52 M) and bubbling for 3 hours. The resulting resin was again washed, yielding $HOOC(CH_2)_2CONH$-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-L-Glu-(O^tBu)-Wang resin. 0.6 g of $ReO(PPh_3)_2Cl_3$ (8 eq.) and 0.32 g of sodium acetate (final 1 M) were added to the resin solution, and the solution heated at 70°C for 2 hours. After cooling to room temperature, the resin was washed and dried, and 3 mL of a TFA "cocktail" (5% water, 5% TIPS, 5% thioanisole and 85% TFA) was added. The solution was allowed to stand for 3 hours. The resin was then filtered and washed once with 1 mL of TFA. Cold ether was added to the collected TFA solution, and the resulting precipitate was washed with cold ether and dried under high vacuum. The resulting mixture was a gray colored solid that weighed 40 mg, a yield of approximately 56 %, and which contained equal quantities of $HOOC(CH_2)_2CONH$-L-Asn-(Trt)-L-Gln-(Trt)-L-Cys-L-Glu (SEQ ID NO:2); $HOOC(CH_2)_2CONH$-L-Asn-(Trt)-D-Gln-(Trt)-L-Cys-L-Glu; $HOOC(CH_2)_2CONH$-D-Asn-(Trt)-L-Gln-(Trt)-L-Cys-L-Glu; and $HOOC(CH_2)_2CONH$-D-Asn-(Trt)-D-Gln-(Trt)-L-Cys-L-Glu.

<u>Example 2</u> ALTERNATE SINGLE-POT SYNTHESIS OF FOUR MEMBER N_3S_1 TYPE METALLOPEPTIDE COMPOUND LIBRARY

1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) as alternative base, replacing sodium acetate. The peptide $HOOC(CH_2)_2CONH$-L-Asn-(Trt)-L-Gln-(Trt)-L-Cys-L-Glu-O^tBu (SEQ ID NO:2)attached to Wang resin was mixed with 1,8-diazabicyclo(5,4,0]undec-7-ene (DBU) (8 eq.) and $ReO(PPh_3)_2Cl_3$ (8 eq.) in DMF. The reaction was carried out at room temperature for 4 hours. The subsequent cleavage of product from the resin, washing and precipitation was as described for Example 1 above.

<u>Example 3</u> IN SITU FORMATION OF METALLO-COMPLEXES IN THE PRESENCE OF REDUCING AGENT AND $ReO(PPh_3)_2Cl_3$

The resin $HOOC(CH_2)_2CONH$-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang obtained from Example 1 above was mixed with tributylphosphine (0.52 M) and $ReO(PPh_3)_2Cl_3$ (8 eq.) in DMF. The bases used in the reaction were either sodium acetate (0.1 M) or 1,8-diazabicyclo(5,4,0]undec-7-ene (DBU) (8 eq.). In the case of sodium acetate, the reaction was

conducted at about 70°C for 4 hours. In the case of DBU, the reaction container was shaken at room temperature for 4 hours. The subsequent cleavage of product from the resin, washing and precipitation was as described for Example 1 above.

Example 4 SYNTHESIS OF Ac-His-X-Cys-Trp-NH_2 (SEQ ID NO:3)— RHENIUM COMPLEXES WITH PEG RESIN (WHERE X = Trp, Homophe, 2-Nal, or Phenylglycine)

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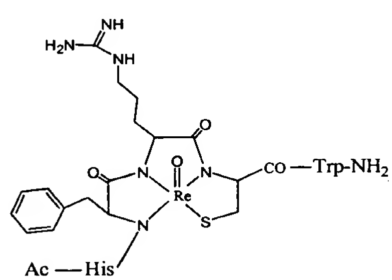
The procedures were similar to those described for Examples 1, 2 and 3. A NovaSyn TGR resin was used. Histidine, Cysteine and Tryptophan were protected by trityl, thio-t-butyl and Boc groups, respectively. The cleavage cocktail was TFA/TIS (95/5). After three hours the resin was filtered and washed with TFA. To the TFA solution was added cold ether, and the resulting precipitate was spun down by centrifugation. The resulting pellets were washed with ether twice, and 0.5 mL of 95% acetic acid was added. Five mL of water was added after one hour. The resulting product was then lyophilized under high vacuum.

Example 5 DEVELOPMENT OF A PROTOTYPE METALLOPEPTIDE LIBRARY FOR THE MELANOCORTIN RECEPTOR.

The library design was based on the tetrapeptide message sequence, His-Phe-Arg-Trp (SEQ ID NO:4) (6-9 sequence), of α-MSH. This sequence exists as a reverse turn, making it suitable for conversion into a metallopeptide format of this invention. In this approach metallopeptides were designed around a tripeptide N_3S_1 MBD designed for a rhenium metal ion. The MBD was derivatized to yield the pentapeptide Ac-His-Phe-Arg-Cys-Trp-NH_2 (SEQ ID NO:5) as a putative candidate for melanocortin ("MC") receptors. Further refinements in the structure were made in response to other considerations, including the chirality of amino acid side chains, yielding a template structure Ac-His-D-Phe-Arg-Cys-Trp-NH_2. The structure of this peptide after binding to rhenium is:



The template structure was used to define a small combinatorial library utilizing split synthesis methodologies. The final template selected for the combinatorial library was Ac-D-His-Xaa-D-Cys-Trp-NH_2, where Xaa was D-(2') Naphthylalanine, D-Trp, D-HomoPhe, or D-Phenylglycine. For this library, the peptide resin, Cys(S^tBu)-Trp(Boc)-Resin was split in four equal parts. Each part was reacted with one of the four Xaa types. After coupling, the resin pools were mixed and synthesis continued in a

Phenylglycine. The peptide resin Cys(Bu^t)-Trp-NH_2 was split into four equal pools and one of the Xaa amino acids was coupled to one individual pool. After completion of the coupling reaction, the four resin pools were mixed again. The synthesis proceeded with the coupling of His followed by acetylation of the N-terminus. After the complete assembly of the peptide chain Ac-His(Trt)-Xaa-Cys(S-Bu^t)-Trp(Boc)-NH_2, the S-Bu^t OSPG group was removed by treatment with DMF/tributylphosphine and rhenium-oxo metal ion was complexed as generally described in Example 1. The fully protected metallopeptide was deblocked and liberated from the solid support by treatment with a cleavage cocktail (95:5 mixture of trifluoroacetic acid - triisopropylsilane) for three hours. The metallopeptide library was recovered by precipitation using cold ether. The resulting pellet was washed twice and 0.5 ml of 95% acetic acid was added. After one-half hour 5 ml of water was added and the solution was freeze-dried yielding the desired library in solid form. Mass spectrometric analysis of the library pool confirmed the correct masses for all four members of the library:

B4

Compound	Structure	Calculated Mass	Mass (M+1) found
1	Ac-His-Phg-Cys-Trp-NH_2 (SEQ ID NO:6)	815.7 and 817.6	815.2 and 816.7
2	Ac-His-Trp-Cys-Trp-NH_2 (SEQ ID NO:7)	868.8 and 870.7	868.0 and 870.1
3	Ac-His-HPhe-Cys-Trp-NH_2 (SEQ ID NO:8)	843.8 and 845.7	842.8 and 845.2
4	Ac-His-2'Nal-Cys-Trp-NH_2 (SEQ ID NO:9)	880.0 and 881.9	879.1 and 880.9

As noted in the table, two molecular ion peaks differing in mass units of 2 were calculated and observed for each structure; this difference is presumptively due to the presence of two natural isotopes of rhenium, Re-185 and Re-187, in the complexation step. In addition, the area under the observed peaks in the spectrometric analysis showed that for each structure the area was in a 1:2 ratio, which is identical to and presumptively related to the relative abundance of Re-185 and Re-187 isotopes. These results confirmed the complexation of rhenium to the peptides. Figure 3 depicts the mass spectrum of a library pool of 4 metallopeptides. The relative intensities of these peaks is due to the differential ionization of individual compounds in the pool and does not reflect the relative amounts in the pool. The spectral analysis did not reveal any free uncomplexed linear peptides, which would be approximately 197 to 199 mass units less than the corresponding metallopeptide, due to the absence of the rhenium-oxo core. Figure 3 depicts reversed phased HPLC profiles of a library pool of 4 metallopeptides. The pool is shown in Figure 4A, and each of the individual peptides is shown in Figures 4B through 4E. Each individual peak in Figures 4B through 4E matched HPLC profiles in the pool in Figure 3A, showing the presence of each of the four compounds in the pool. Each individual peptide is resolved into two isomeric peaks (syn- and anti-isomers) that are due to two alternate orientations of the oxygen atom in the Re=O core. All four compounds used for this comparison were

obtained with Leu or Ile in the position Aaa in the peptide chain. The peptide Z-Leu-Ser-Cys-Val-NH_2 (SEQ ID NO:10) displayed an IC_{50} value of 139 μM and the peptide Z-Ile-Ser-Cys-Val-NH_2 (SEQ ID NO:11) displayed an IC_{50} value of 179 μM. Substitution of the three residues Ala, Phe, and Lys(N^{ϵ}-Z) at Aaa yielded IC_{50} values in excess of 1,000 μM. Based on these results, the two peptides Z-Leu-Ser-Cys-Val-NH_2 (SEQ ID NO:10) and Z-Ile-Ser-Cys-Val-NH_2 (SEQ ID NO:11) appeared to meet stereochemical requirements as an HNE inhibitor. The results of this assay, where Z is benzyloxycarbonyl, are shown in the following table.

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R	Aaa	Bbb	IC_{50} (μM)
Z	Ala	Ser	> 1000
Z	Leu	Ser	139
Z	Ile	Ser	179
Z	Phe	Ser	>1000
Z	Lys(Nϵ-Z)	Ser	>1000

Example 13 SYNTHESIS OF -SH CONTAINING BUILDING BLOCKS FROM HALOGENATED COMPOUNDS.

A variety of "S" containing building blocks (other than amino acids) for use in the synthesis of libraries according to this invention can be synthesized from corresponding halogenated congeners. The process relies on the treatment of a halogenated compound with sodium thiosulfate at slightly basic pH to obtain the corresponding Bunte salt (the S-sulfonate derivative) as described by Wunderlin R et al: *Helv Chim Acta* 68:12-22, 1985. The Bunte salt S-sulfonate derivative is treated with a reducing agent such as 2-mercaptoethane, sodium borohydride or tributyl phosphine to yield a free thiol-containing product. This product can then be S-protected with conventional S-protecting groups such as Trt, mmt, Npys, Bu^t, S-Bu^t known in the art of peptide synthesis. Alternatively, and preferably in this invention, the Bunte salt S-sulfonate derivative may be directly used in the synthesis of peptides as described by Maugras I et al: *Int J Peptide Protein Res* 45:152, 1995. The S-sulfonate derivative is stable using either Fmoc or Boc synthetic peptide synthesis methods, and following peptide synthesis the resulting peptide may be treated with tributyl phosphine to liberate the free -SH group. In either method, the resulting peptide has an available free thiol for complexation with a metal ion, which complexation may be according to either the solid phase or solution phase methods described above.

Example 14 SYNTHESIS OF REDUCED PEPTIDE BOND METALLOPEPTIDE LIBRARY

Metallopeptide libraries may be synthesized wherein the library members contain either a reduced peptide bond or an N-substituted reduced peptide bond, such that the library members contain a CH_2-NH group or CH_2-NR group rather than a CO-NH peptide bond. Examples of these structures are given at Figure 1A, 1D, 1E, 1H and 1J. The synthetic methods employed are similar to those for

subsequent to metal ion complexation the remaining protecting groups are liberated to yield the desired metallo-peptide complex.

A fully protected peptide-resin containing two S-protected thiol groups (Cys residues), Fmoc-Tyr(Bu^t)-Cys(Trt)-Gly-Phe-Cys(S^{t}Bu)-Wang resin, was prepared by standard solid-phase peptide synthesis means. The N-terminal Fmoc-group was removed by treatment with 20% piperidine in DMF to give the resin Tyr(Bu^t)-Cys(Trt)-Gly-Phe-Cys(S^{t}Bu)-Wang resin. In this case, the Cys(Trt) group and the Cys(S^{t}Bu) protecting groups are orthogonal to each other. While the Cys(Trt) is an acid labile group, the Cys(S^{t}Bu) group may be removed under reductive conditions, thereby allowing selective deprotection of one thiol group. The peptide-resin was treated with tributylphosphine (0.52 M) in DMF to remove the S-S^{t}Bu group from the Cys residue. The resin was then treated with $Re(O)Cl_3(PPh_3)_2$ (8 eq.) in the presence of DBU as base for 4 hours at room temperature to complete the formation of the ReO[V] complex with the peptide. The peptide resin was washed extensively, dried and treated with TFA/TIS (95:5) cleavage cocktail to yield the metallo-peptide. The metallo-peptide was precipitated using MeOH-ether, and the product was dried and purified by HPLC. The purified peptide was analyzed by electron spray mass spectrometry, yielding predicted mass for the metallo-peptide complex.

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Example 19 SYNTHESIS OF A METALLO-PEPTIDE LIBRARY CONTAINING TWO “S” GROUPS BUT UTILIZING ONLY ONE “S” FOR SITE SPECIFIC COMPLEXATION OF METAL ION TO A PEPTIDE CHAIN BY ORTHOGONAL PROTECTION OF SULFYDRYL GROUPS IN THE PEPTIDE SEQUENCES. SYNTHESIS OF A LIBRARY WITH GENERAL STRUCTURE: Tyr-Cys-[Aaa-Phe-Cys]-ReO[V]

A library of metallo-peptides containing two “S” capable of complexing with ReO[V] core, but directing this bond formation with only one of these two “S” atoms can be synthesized as set forth above. Fully protected peptide resin Fmoc-Tyr(Bu^t)-Cys(Trt)-Aaa-Phe-Cys(S^{t}Bu) (SEQ ID NO:12)-Wang resin is prepared by solid-phase methods of peptide synthesis. The “Aaa” is an alpha amino amino acid, synthetic or naturally occurring in either L- or D-isomeric form, as may be desired for the composition of individual library members. Individual compounds with different Aaa group may be synthesized in parallel for the synthesis of a parallel library of compounds. Alternatively, all compounds may be synthesized as a mixture in one pot using different “Aaa” building blocks at the coupling step for Aaa. Alternatively, the library mixture may also be synthesized by a split and pool approach as described above using various Aaa groups.

The finished peptide-resin is treated with 20% piperidine to remove the terminal Fmoc group. Treatment of the Tyr(Bu^t)-Cys(Trt)-Gly-Phe-Cys(S^{t}Bu) (SEQ ID NO:13)-Wang resin thus obtained with tributylphosphine and $Re(O)Cl_3(PPh_3)_2$ in the presence of DBU can be accomplished as described above to complex the ReO metal ion specifically at the C-terminal Cys residue while leaving the Cys(Trt) residue inert.

throughout the specification and in the claims. The primary applications of this invention involve human patients, but this invention may be applied to laboratory, farm, zoo, wildlife, pet, sport or other animals. The products of this invention may optionally employ radionuclide ions, which may be used for diagnostic imaging purposes or for radiotherapeutic purposes.

Peptide and Metallo-Construct Molecule Libraries and Combinatorial Chemistries. Using the methods of this invention, libraries of peptides and peptidomimetics are designed wherein each constituent library member includes an MBD sequence necessary for providing a coordination site for complexation with a metal, it being understood that such sequence may differ among the constituent members of the library. Upon complexing the MBD with a metal, a specific structure results, forming a mimic of a reverse turn structure. The specific stereochemical features of this complex are due to the stereochemistry of the coordination sphere of the complexing metal ion. Thus the preferred geometry of the coordination sphere of the metal dictates and defines the nature and extent of conformational restriction.

Libraries of this invention contain constituents which are either locally or globally constrained structures. Libraries may include molecules with either local conformation restrictions or global conformation restrictions, or some combination thereof. This aspect of the invention includes a variety of methods of synthesis, screening and structural elucidation of positive hits in screening systems. The importance of these aspects are well known to those skilled in the art and will also become evident from the following description and examples.

In general, most of the metals that may prove useful in this invention have a coordination number of 4 to 6, and rarely as high as 8, which implies that the putative MBD must be made of residues with reactive groups located in a stereocompatible manner so as to establish a bond with a metal ion of given geometry and coordination sphere. Coordinating groups in the peptide chain include nitrogen atoms of amine, amide, imidazole, or guanidino functionalities; sulfur atoms of thiols or disulfides; and oxygen atoms of hydroxy, phenolic, carbonyl, or carboxyl functionalities. In addition, the peptide chain or individual amino acids can be chemically altered to include a coordinating group, such as oxime, hydrazino, sulfhydryl, phosphate, cyano, pyridino, piperidino, or morpholino groups. For a metal with a coordination number of 4, a tetrapeptide amino acid sequence may be employed (such as Gly-Gly-Gly-Gly) (SEQ ID NO:1), or a tripeptide amino acid sequence in which at least one of the amino acids has a side chain with a coordinating group can similarly be employed (such as Gly-Gly-Cys). The side chain can have a nitrogen, oxygen or sulfur-based coordination group. Thus, an amino acid sequence can provide an N_4, N_3S, N_2S_2, NS_3, N_2SO or similar ligand, yielding tetradentate coordination of a metal ion utilizing nitrogen, sulfur and oxygen atoms.

In another embodiment of the invention, the MBD includes one or more amino acid residues and one or more derivatized amino acids or spacer sequences, with the derivatized amino acid or spacer

six equivalent of diisopropylethylamine (DIEA) were added with NMP and bubbled with nitrogen for 30 min. The resin was again washed and deprotection of the Fmoc-group repeated, yielding NH_2-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. The resin was then split into two pools, and using similar methods Fmoc-L-Gln-(Trt)-OH was added to one pool and Fmoc-D-Gln-(Trt)-OH added to the other pool. The two pools were mixed and the Fmoc-group deprotected, yielding NH_2-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. The resin was again split into two pools, and Fmoc-L-Asn-(Trt)-OH added to one pool and Fmoc-D-Asn-(Trt)-OH added to the other pool. The two pools were mixed and the Fmoc-group deprotected, yielding NH_2-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. To this resin was added 0.072 g of succinic anhydride in pyridine, yielding $HOOC(CH_2)_2$CONH-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang resin. After washing, the S^tBu group was removed by adding 13.8 mL of DMF/Tributylphosphine (20/3, v/v; 0.52 M) and bubbling for 3 hours. The resulting resin was again washed, yielding $HOOC(CH_2)_2$CONH-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-L-Glu-(O^tBu)-Wang resin. 0.6 g of $ReO(PPh_3)_2Cl_3$ (8 eq.) and 0.32 g of sodium acetate (final 1 M) were added to the resin solution, and the solution heated at 70°C for 2 hours. After cooling to room temperature, the resin was washed and dried, and 3 mL of a TFA "cocktail" (5% water, 5% TIPS, 5% thioanisole and 85% TFA) was added. The solution was allowed to stand for 3 hours. The resin was then filtered and washed once with 1 mL of TFA. Cold ether was added to the collected TFA solution, and the resulting precipitate was washed with cold ether and dried under high vacuum. The resulting mixture was a gray colored solid that weighed 40 mg, a yield of approximately 56 %, and which contained equal quantities of $HOOC(CH_2)_2$CONH-L-Asn-(Trt)-L-Gln-(Trt)-L-Cys-L-Glu (SEQ ID NO:2); $HOOC(CH_2)_2$CONH-L-Asn-(Trt)-D-Gln-(Trt)-L-Cys-L-Glu; $HOOC(CH_2)_2$CONH-D-Asn-(Trt)-L-Gln-(Trt)-L-Cys-L-Glu; and $HOOC(CH_2)_2$CONH-D-Asn-(Trt)-D-Gln-(Trt)-L-Cys-L-Glu.

Example 2 ALTERNATE SINGLE-POT SYNTHESIS OF FOUR MEMBER N_3S_1 TYPE METALLOPEPTIDE COMPOUND LIBRARY

1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) as alternative base, replacing sodium acetate. The peptide $HOOC(CH_2)_2$CONH-L-Asn-(Trt)-L-Gln-(Trt)-L-Cys-L-Glu-O^tBu (SEQ ID NO:2)attached to Wang resin was mixed with 1,8-diazabicyclo(5,4,0]undec-7-ene (DBU) (8 eq.) and $ReO(PPh_3)_2Cl_3$ (8 eq.) in DMF. The reaction was carried out at room temperature for 4 hours. The subsequent cleavage of product from the resin, washing and precipitation was as described for Example 1 above.

Example 3 IN SITU FORMATION OF METALLO-COMPLEXES IN THE PRESENCE OF REDUCING AGENT AND $ReO(PPh_3)_2Cl_3$

The resin $HOOC(CH_2)_2$CONH-(L,D)-Asn-(Trt)-(L,D)-Gln-(Trt)-L-Cys-(S^tBu)-L-Glu-(O^tBu)-Wang obtained from Example 1 above was mixed with tributylphosphine (0.52 M) and $ReO(PPh_3)_2Cl_3$ (8 eq.) in DMF. The bases used in the reaction were either sodium acetate (0.1 M) or 1,8-diazabicyclo(5,4,0]undec-7-ene (DBU) (8 eq.). In the case of sodium acetate, the reaction was

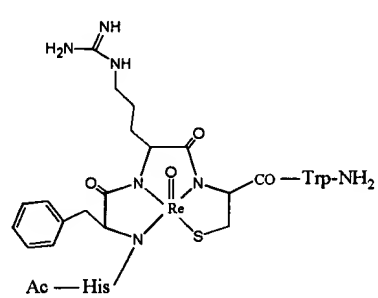
conducted at about 70 °C for 4 hours. In the case of DBU, the reaction container was shaken at room temperature for 4 hours. The subsequent cleavage of product from the resin, washing and precipitation was as described for Example 1 above.

Example 4 SYNTHESIS OF Ac-His-X-Cys-Trp-NH_2 (SEQ ID NO:3)— RHENIUM COMPLEXES WITH PEG RESIN (WHERE X = Trp, Homophe, 2-Nal, or Phenylglycine)

The procedures were similar to those described for Examples 1, 2 and 3. A NovaSyn TGR resin was used. Histidine, Cysteine and Tryptophan were protected by trityl, thio-t-butyl and Boc groups, respectively. The cleavage cocktail was TFA/TIS (95/5). After three hours the resin was filtered and washed with TFA. To the TFA solution was added cold ether, and the resulting precipitate was spun down by centrifugation. The resulting pellets were washed with ether twice, and 0.5 mL of 95% acetic acid was added. Five mL of water was added after one hour. The resulting product was then lyophilized under high vacuum.

Example 5 DEVELOPMENT OF A PROTOTYPE METALLOPEPTIDE LIBRARY FOR THE MELANOCORTIN RECEPTOR.

The library design was based on the tetrapeptide message sequence, His-Phe-Arg-Trp (SEQ ID NO:4) (6-9 sequence), of α-MSH. This sequence exists as a reverse turn, making it suitable for conversion into a metallopeptide format of this invention. In this approach metallopeptides were designed around a tripeptide N_3S_1 MBD designed for a rhenium metal ion. The MBD was derivatized to yield the pentapeptide Ac-His-Phe-Arg-Cys-Trp-NH_2 (SEQ ID NO:5) as a putative candidate for melanocortin ("MC") receptors. Further refinements in the structure were made in response to other considerations, including the chirality of amino acid side chains, yielding a template structure Ac-His-D-Phe-Arg-Cys-Trp-NH_2. The structure of this peptide after binding to rhenium is:



The template structure was used to define a small combinatorial library utilizing split synthesis methodologies. The final template selected for the combinatorial library was Ac-D-His-Xaa-D-Cys-Trp-NH_2, where Xaa was D-(2') Naphthylalanine, D-Trp, D-HomoPhe, or D-Phenylglycine. For this library, the peptide resin, Cys(S^tBu)-Trp(Boc)-Resin was split in four equal parts. Each part was reacted with one of the four Xaa types. After coupling, the resin pools were mixed and synthesis continued in a

Phenylglycine. The peptide resin Cys(Bu^t)-Trp-NH_2 was split into four equal pools and one of the Xaa amino acids was coupled to one individual pool. After completion of the coupling reaction, the four resin pools were mixed again. The synthesis proceeded with the coupling of His followed by acetylation of the N-terminus. After the complete assembly of the peptide chain Ac-His(Trt)-Xaa-Cys(S-Bu^t)-Trp(Boc)-NH_2, the S-Bu^t OSPG group was removed by treatment with DMF/tributylphosphine and rhenium-oxo metal ion was complexed as generally described in Example 1. The fully protected metallopeptide was deblocked and liberated from the solid support by treatment with a cleavage cocktail (95:5 mixture of trifluoroacetic acid - triisopropylsilane) for three hours. The metallopeptide library was recovered by precipitation using cold ether. The resulting pellet was washed twice and 0.5 ml of 95% acetic acid was added. After one-half hour 5 ml of water was added and the solution was freeze-dried yielding the desired library in solid form. Mass spectrometric analysis of the library pool confirmed the correct masses for all four members of the library:

Compound	**Structure**	**Calculated Mass**	**Mass (M+1) found**
1	Ac-His-Phg-Cys-Trp-NH_2 (SEQ ID NO:6)	815.7 and 817.6	815.2 and 816.7
2	Ac-His-Trp-Cys-Trp-NH_2 (SEQ ID NO:7)	868.8 and 870.7	868.0 and 870.1
3	Ac-His-HPhe-Cys-Trp-NH_2 (SEQ ID NO:8)	843.8 and 845.7	842.8 and 845.2
4	Ac-His-2'Nal-Cys-Trp-NH_2 (SEQ ID NO:9)	880.0 and 881.9	879.1 and 880.9

As noted in the table, two molecular ion peaks differing in mass units of 2 were calculated and observed for each structure; this difference is presumptively due to the presence of two natural isotopes of rhenium, Re-185 and Re-187, in the complexation step. In addition, the area under the observed peaks in the spectrometric analysis showed that for each structure the area was in a 1:2 ratio, which is identical to and presumptively related to the relative abundance of Re-185 and Re-187 isotopes. These results confirmed the complexation of rhenium to the peptides. Figure 3 depicts the mass spectrum of a library pool of 4 metallopeptides. The relative intensities of these peaks is due to the differential ionization of individual compounds in the pool and does not reflect the relative amounts in the pool. The spectral analysis did not reveal any free uncomplexed linear peptides, which would be approximately 197 to 199 mass units less than the corresponding metallopeptide, due to the absence of the rhenium-oxo core. Figure 3 depicts reversed phased HPLC profiles of a library pool of 4 metallopeptides. The pool is shown in Figure 4A, and each of the individual peptides is shown in Figures 4B through 4E. Each individual peak in Figures 4B through 4E matched HPLC profiles in the pool in Figure 3A, showing the presence of each of the four compounds in the pool. Each individual peptide is resolved into two isomeric peaks (syn- and anti-isomers) that are due to two alternate orientations of the oxygen atom in the Re=O core. All four compounds used for this comparison were

obtained with Leu or Ile in the position Aaa in the peptide chain. The peptide Z-Leu-Ser-Cys-Val-NH_2 (SEQ ID NO:10)displayed an IC_{50} value of 139 μM and the peptide Z-Ile-Ser-Cys-Val-NH_2 (SEQ ID NO:11)displayed an IC_{50} value of 179 μM. Substitution of the three residues Ala, Phe, and Lys(N^{ϵ}-Z) at Aaa yielded IC_{50} values in excess of 1,000 μM. Based on these results, the two peptides Z-Leu-Ser-Cys-Val-NH_2 (SEQ ID NO:10) and Z-Ile-Ser-Cys-Val-NH_2 (SEQ ID NO:11) appeared to meet stereochemical requirements as an HNE inhibitor. The results of this assay, where Z is benzyloxycarbonyl, are shown in the following table.

R	**Aaa**	**Bbb**	**IC_{50} (μM)**
Z	Ala	Ser	> 1000
Z	Leu	Ser	139
Z	Ile	Ser	179
Z	Phe	Ser	>1000
Z	Lys(Nϵ-Z)	Ser	>1000

Example 13 SYNTHESIS OF -SH CONTAINING BUILDING BLOCKS FROM HALOGENATED COMPOUNDS.

A variety of "S" containing building blocks (other than amino acids) for use in the synthesis of libraries according to this invention can be synthesized from corresponding halogenated congeners. The process relies on the treatment of a halogenated compound with sodium thiosulfate at slightly basic pH to obtain the corresponding Bunte salt (the S-sulfonate derivative) as described by Wunderlin R et al: *Helv Chim Acta* 68:12-22, 1985. The Bunte salt S-sulfonate derivative is treated with a reducing agent such as 2-mercaptoethane, sodium borohydride or tributyl phosphine to yield a free thiol-containing product. This product can then be S-protected with conventional S-protecting groups such as Trt, mmt, Npys, Bu^t, S-Bu^t known in the art of peptide synthesis. Alternatively, and preferably in this invention, the Bunte salt S-sulfonate derivative may be directly used in the synthesis of peptides as described by Maugras I et al: *Int J Peptide Protein Res* 45:152, 1995. The S-sulfonate derivative is stable using either Fmoc or Boc synthetic peptide synthesis methods, and following peptide synthesis the resulting peptide may be treated with tributyl phosphine to liberate the free -SH group. In either method, the resulting peptide has an available free thiol for complexation with a metal ion, which complexation may be according to either the solid phase or solution phase methods described above.

Example 14 SYNTHESIS OF REDUCED PEPTIDE BOND METALLOPEPTIDE LIBRARY

Metallopeptide libraries may be synthesized wherein the library members contain either a reduced peptide bond or an N-substituted reduced peptide bond, such that the library members contain

a CH_2-NH group or CH_2-NR group rather than a CO-NH peptide bond. Examples of these structures are given at Figure 1A, 1D, 1E, 1H and 1J. The synthetic methods employed are similar to those for

subsequent to metal ion complexation the remaining protecting groups are liberated to yield the desired metallo-peptide complex.

A fully protected peptide-resin containing two S-protected thiol groups (Cys residues), Fmoc-Tyr(Bu^t)-Cys(Trt)-Gly-Phe-Cys(S^tBu)-Wang resin, was prepared by standard solid-phase peptide synthesis means. The N-terminal Fmoc-group was removed by treatment with 20% piperidine in DMF to give the resin Tyr(Bu^t)-Cys(Trt)-Gly-Phe-Cys(S^tBu)-Wang resin. In this case, the Cys(Trt) group and the Cys(S^tBu) protecting groups are orthogonal to each other. While the Cys(Trt) is an acid labile group, the Cys(S^tBu) group may be removed under reductive conditions, thereby allowing selective deprotection of one thiol group. The peptide-resin was treated with tributylphosphine (0.52 M) in DMF to remove the S-S^tBu group from the Cys residue. The resin was then treated with $Re(O)Cl_3(PPh_3)_2$ (8 eq.) in the presence of DBU as base for 4 hours at room temperature to complete the formation of the ReO[V] complex with the peptide. The peptide resin was washed extensively, dried and treated with TFA/TIS (95:5) cleavage cocktail to yield the metallo-peptide. The metallo-peptide was precipitated using MeOH-ether, and the product was dried and purified by HPLC. The purified peptide was analyzed by electron spray mass spectrometry, yielding predicted mass for the metallo-peptide complex.

<u>Example 19</u> SYNTHESIS OF A METALLO-PEPTIDE LIBRARY CONTAINING TWO "S" GROUPS BUT UTILIZING ONLY ONE "S" FOR SITE SPECIFIC COMPLEXATION OF METAL ION TO A PEPTIDE CHAIN BY ORTHOGONAL PROTECTION OF SULFYDRYL GROUPS IN THE PEPTIDE SEQUENCES. SYNTHESIS OF A LIBRARY WITH GENERAL STRUCTURE: Tyr-Cys-[Aaa-Phe-Cys]-ReO[V]

A library of metallo-peptides containing two "S" capable of complexing with ReO[V] core, but directing this bond formation with only one of these two "S" atoms can be synthesized as set forth above. Fully protected peptide resin Fmoc-Tyr(Bu^t)-Cys(Trt)-Aaa-Phe-Cys(S^tBu) (<u>SEQ ID NO:12</u>)-Wang resin is prepared by solid-phase methods of peptide synthesis. The "Aaa" is an alpha amino amino acid, synthetic or naturally occurring in either L- or D-isomeric form, as may be desired for the composition of individual library members. Individual compounds with different Aaa group may be synthesized in parallel for the synthesis of a parallel library of compounds. Alternatively, all compounds may be synthesized as a mixture in one pot using different "Aaa" building blocks at the coupling step for Aaa. Alternatively, the library mixture may also be synthesized by a split and pool approach as described above using various Aaa groups.

The finished peptide-resin is treated with 20% piperidine to remove the terminal Fmoc group. Treatment of the Tyr(Bu^t)-Cys(Trt)-Gly-Phe-Cys(S^tBu) (<u>SEQ ID NO:13</u>)-Wang resin thus obtained with tributylphosphine and $Re(O)Cl_3(PPh_3)_2$ in the presence of DBU can be accomplished as described above to complex the ReO metal ion specifically at the C-terminal Cys residue while leaving the Cys(Trt) residue inert.